Table S1. Structure refinement statistics.

	Dark used in I_{120K}	**Dark2 used in I_{180K}**	**Dark (W285F)**
Crystal ID	WT-15	WT-32	W285F-01
R-factor	0.147	0.149	0.152
Free R-factor	0.181	0.182	0.184
Resolution (Å)	1.67	1.61	1.75
# of reflections	134,809	165,032	127,771
Contents in ASU	4 molecules + 1600 waters + 20 Mg ion		
RMSD (bond length) (Å)	0.006	0.006	0.006
RMSD (bond angle) (°)	1.046	1.047	1.054
Cell parameters	112.5 77.3 189.3 (Å) 90.0 95.6 90.0 (°)	113.3 76.8 191.3 (Å) 90.0 97.0 90.0(°)	111.8 79.1 189.3 (Å) 90.00 95.2 90.0(°)
PDB accession ID*	4NAA	4NBM	4NC4

Instruction for PDB users:

The coordinates of each dark reference structure along with its associated structure factor amplitudes of the dark and UV data sets (collected from the same crystal) as well as their difference amplitudes are deposited in Protein Data Bank under the same accession number. Users are advised to download both the coordinates and structural factors to calculate the (F_{UV}-F_{Dark}) difference map for proper examination of light-induced structural changes at each temperature.

Table S2. Data collection statistics in T-scan and control experiments.

Crystal ID	**WT-20**		**WT-33**		**WT-15**		**WT-16**		**WT-21**		**WT-22**		**WT-32***		**WT-41 (control)**			**W285F**	
Temperature	100K		110K		120K		140K		160K		180K		180K			120 K	180 K	120K	
	DK	UV	DK	UV	DK	UV	DK	UV	DK	UV	DK	UV	DK	UV	DK	NO UV		DK	UV
Resolution (Å)	1.75	1.75	1.70	1.70	1.67	1.73	1.75	1.9	1.90	1.9	1.9	1.9	1.61	1.8	1.8	1.85	1.85	1.75	1.90
Rmerge (%)	4.4	4.2	4.9	4.6	3.9	4.8	5.5	6.8	6.0	6.4	6.5	5.4	3.8	4.3	8.1	12.6	5.1	4.7	7.5
Completeness (%)	86.7	86.8	87.0	80.5	82.8	82.5	97.3	97.9	98.8	98.5	98.4	91.6	87.9	87.6	95.5	90.3	87.6	99.8	99.5
R-diff (DK vs. DK)[1]	0.22		0.34		Ref		0.09		0.11		0.11		0.34		0.25			0.19	
R-diff (DK vs.UV)[2]	0.049		0.055		0.072		0.124		0.086		0.189		0.169			0.08	0.21	0.084	
Redundancy	4.6	4.6	5.2	5.2	4.6	4.6	2.9	3.1	4.9	4.9	4.4	4.2	4.4	4.5	3.0	2.9	2.7	3.6	3.6

*Crystal WT-32 exhibits slightly different cell parameters and was used to refine the I_{180K} structure. See also Fig. S2c and Table S2.

[1] R-diff (Dark vs. Dark) refers to the difference R-factor between two dark sets from different crystals calculated by ScaleIt in CCP4. Crystal WT-15 was used as the reference.

[2] R-diff (Dark vs. UV) refers to the difference R-factor between the dark and UV data sets from different volumes of the same crystal calculated by ScaleIt in CCP4.

Table S3. **Time-resolved fluorescence decay experiments**. Lifetimes (τ) of distinct fluorescence decay species and their relative populations (α_i) extracted from 3-expontial fitting of time-resolved data from WT and W285F (10 protein samples) *At*UVR8 samples. The excitation and emission wavelengths were set at 320nm and 350nm, respectively. See SI Methods for experimental and statistical details. Time-courses from 10 independent samples show good agreement in both WT and W285F. χ is residual of the least-squares fitting procedure.

WT

Sample	τ_1（ns）	α_1	τ_2（ns）	α_2	τ_3（ns）	α_3	χ^2
1	0.076	77.3%	1.51	16.3%	8.00	6.4%	1.014
2	0.072	77.2%	1.45	15.8%	8.00	7.0%	1.048
3	0.072	78.8%	1.64	15.8%	9.52	5.4%	1.067
4	0.068	78.4%	1.53	15.1%	7.93	6.5%	1.010
5	0.051	78.5%	1.42	15.0%	7.42	6.5%	1.093
6	0.067	78.3%	1.49	15.2%	8.18	6.5%	1.082
7	0.092	77.6%	1.56	15.7%	8.96	6.7%	1.095
8	0.061	78.0%	1.43	15.3%	7.43	6.7%	1.081
9	0.050	81.1%	1.55	13.0%	7.83	5.9%	1.080
10	0.063	79.2%	1.53	14.6%	8.84	6.2%	1.036

W285F

Sample	τ_1（ns）	α_1	τ_2（ns）	α_2	τ_3（ns）	α_3	χ^2
1	0.055	4.2%	2.88	1.5%	12.92	94.3%	1.124
2	0.036	4.6%	4.10	2.1%	12.99	92.3%	1.142
3	0.051	3.8%	2.20	1.2%	12.79	95.0%	1.066
4	0.039	4.6%	2.75	1.6%	12.91	93.8%	1.075
5	0.081	3.3%	4.19	2.5%	12.98	94.2%	1.154
6	0.028	5.5%	3.73	2.1%	12.97	92.4%	1.123
7	0.053	3.8%	3.79	2.5%	13.02	93.7%	1.082
8	0.065	3.6%	3.02	2.0%	12.98	94.4%	1.158
9	0.041	5.0%	2.02	1.2%	12.84	93.8%	1.088
10	0.042	5.5%	3.05	1.6%	12.89	92.9%	1.047

Table S4. Geometric parameters of 13 Trp residues in the dark structure of *At*UVR8.

	Subunit A				**Subunit B**				**Subunit C**				**Subunit D**			
	Side chain		Main chain		Side chain		Main chain		Side chain		Main chain		Side chain		Main chain	
Torsional angles (°)	χ1	χ2	ϕ	ψ	χ1	χ2	ϕ	ψ	χ1	χ2	ϕ	ψ	χ1	χ2	ϕ	ψ
Group 1 (G/S-**W-G)**																
Trp39	64	96	-159	165	61	99	-159	165	61	97	-159	167	62	99	-160	165
Trp92	59	98	-162	161	57	100	-162	161	57	97	-161	161	59	98	-163	162
Trp144	62	96	-160	157	65	92	-158	159	60	96	-159	160	63	95	-164	160
Trp196	62	102	-160	168	67	99	-162	167	63	99	-159	168	63	101	-161	169
Trp300	57	97	-162	170	56	97	-162	169	58	95	-163	168	57	96	-162	167
Trp352	59	96	-158	163	58	57	-157	161	59	97	-159	163	59	95	-156	161
Group 2 (G-W-G/S/**N)**																
Trp94	-170	1	-66	131	-166	-1	-61	127	-170	0	-66	130	-170	2	-64	131
Trp198	-173	97	-61	124	-175	99	-63	122	-176	103	-62	127	-176	99	-65	123
Trp250	-158	23	-80	131	-153	20	-73	130	-158	26	-77	132	-155	22	-74	132
Trp302	-167	47	-67	135	-165	40	-65	133	-170	49	-68	136	-168	49	-66	134
Group 3 (G-W-R)																
Trp233	-156	55	-61	-47	-152	53	-57	-51	-152	51	-61	-48	-154	54	-61	-52
Trp285	-162	32	-63	-52	-166	35	-64	-54	-161	32	-62	-55	-164	36	-61	-55
Trp337	-80	25	-75	-41	-78	26	-75	-41	-79	27	-72	-40	-79	27	-75	-39